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REMARKS

Claims 29-20 and 32-37 remain pending. Favorable reconsideration is respectfully requested.

The present invention relates to a mouse suitable as a model for atopic dermatitis, wherein the mouse is a NC/Nga mouse which has been sensitized with a mite extract on the ear(s) under a specific pathogen free environment, such that the animal displays at least one symptom of atopic dermatitis caused by the mite extract. See Claim 29.

The present invention also relates to a method of producing the mouse described above, and to a method of screening for an agent for effectiveness against atopic dermatitis using the mouse described above. See Claims 35 and 37, respectively.

The rejections of the claims under 35 U.S.C. §103(a) over Morita et al. in view of Yasue et al., Gad, and Hiroi et al. are respectfully traversed. Those references fail to suggest the claimed mouse, and the claimed methods of making and screening.

Morita et al. describe a mouse that has skin lesions over its whole body. The mouse is prepared by infecting a NC mouse with live fur mites, and, after about four weeks, it demonstrates lesions on the skin which are associated with elevated serum IgE levels. The mouse prepared in this manner is useful as a model for atopic dermatitis. See the Abstract.

As part of the procedure for studying the mice, Morita et al. extracted the fur mites to obtain their proteins for use in assaying serum antigen-specific IgE (see page 38, column 2, section 2.5). In Morita et al. there is no teaching or suggestion to apply the mite extract to the ear(s) of the mouse. Morita et al. describe using live fur mites, not the extract of the mites, in order to produce a mouse that can be used as a model for atopic dermatitis.

Yasue et al. describe a study designed to evaluate the hyposensitizing activity of recombinant Der f 2 (rDer f 2). See the Abstract. In the study, mice were cosensitized with rDer f 2 crude and mite extract, and then challenged with crude mite extract (experiment 2, page 30, second column, first paragraph under the section entitled "Materials and Methods"; see also the section entitled "Experiment 2" at page 32, first column). Yasue et al. report that

> Mite extract inhalation challenge provoked neutophilia in rDer f 2 + mite-sensitized control mice, and this allergic reaction tended to decrease in sensitized mice fed 1 mg/day of rDer f 2 orally for 4 weeks. We conclude that rDer f 2 has hyposensitizing activities and may be useful in immunotherapy for house dust mite allergy. [Abstract, last sentence.]

Thus, in the procedure described by Yasue et al. the crude mite extract is administered by inhalation via an aerosol (see page 32, first column, bottom). As described at page 33, column 2, in the paragraph preceding the "Discussion" section, "inhalation of mite extract provoked late-phase airway inflammation characterized by neutrophil influx in the mice."

Yasue et al. provide a detailed discussion of the biochemical responses provoked in the study described in that reference (Experiment 2, see page 33, second column, bottom to page 36). Significant by its absence is any mention of skin lesions, atopic dermatitis, or IgE.

The Gad reference is a review article describing the mouse ear swelling test (known as "MEST"). This publication provides the current version of the MEST protocol (as of 1994, when the review was published). See the Abstract. Nowhere does Gad discuss using MEST to evaluate the effect of mite extract. Gad is also silent about using MEST to prepare a mouse model for atopic dermatitis.

Claims 29-20 and 32-37 are not suggested by Morita et al. in view of Yasue et al. and Gad. The Examiner admits that Morita et al. (a) describe preparing a mite extract and (b) explicitly teach administering live mites and not the mite extract to the mice (see the Official Action dated March 10, 2004 at page 4, lines 1-3). To overcome this glaring deficiency, the Examiner relies on Yasue et al., stating:

> Yasue et al. supplements Morita by teaching that that the administration of mite extract to generate allergic responses in mice is a standard procedure (Yasue et al., page 30, column 2, and page 32). It is further noted that the skilled artisan would be motivated to use a mite extract over live mites in order to standardize the amount of antigen to which each mouse is exposed, thereby ensuring a homogeneous population of mite sensitized mice for use as an animal model of atopic dermatitis, it would have been *prima facie* obvious to skilled artisan to substitute mite extract sensitization as taught by Yasue et al. for the live mite exposure taught by Morita et al. in the method of producing a murine model of atopic dermatitis taught by Morita et al. Further, based on the successful use of mite extracts to generate allergic responses in mice as taught by Yasue et al., the skilled artisan would have had a reasonable expectation of success in generating a mouse model of atopic dermatitis by exposing specific pathogen free NC mice to a mite extract.

There is no teaching in Yasue et al. that it is a "standard procedure" in the art to administer mite extract to generate allergic responses in mice. Yasue et al. certainly use mite extract as part of their study of rDer f 2, but there is no indication in that reference, or any other reference cited, that the use of mite antigen is part of a "standard procedure" for inducing an immune response in mice.

The Examiner's logic with respect to substituting mite extract for the live mites used by Morita et al. flies in the face of the simple fact that Morita et al. explicitly describe preparing mite extract and using it to assay for IgE, but use live mites to infect the mice in

order to induce dermatitis on the skin of the mice. Thus, the direct teaching of the Morita et al. reference contradicts the Examiner's logic in combining that reference with Yasue et al. Stated another way, if it was so apparent that mite extract was preferable to live mites, then why didn't Morita et al. use the mite extract described in that reference instead of the live mites? Morita et al. must have believed that live mites would be more effective as compared to mite extract because they used the former and not the latter. The Examiner's reasoning that one would be motivated to use mite extract instead of live mites is nothing but hindsight reconstruction of the claimed mouse using the cited references.

The Examiner also relies on Yasue et al. to support the argument that mite extracts are known to "generate allergic responses in mice." However, in the study described by Yasue et al. the mite extract was administered via inhalation; it was not topically applied to the skin. For that reason, there is no suggestion from Yasue et al. that mite extract can be applied topically to the ears of a mouse in order to cause the mouse to display at least one symptom of atopic dermatitis, as claimed.

The Examiner has taken the position that Gad provides "motivation for sensitizing a mouse with an allergen on the ear." However, Gad is completely silent with respect to fur mites or an extract of fur mites. The "test materials" discussed in Gad are "treated fabrics, medical devices, environmental pollutants, specialty chemicals, and drugs" (see the Abstract). Therefore, Gad provides no suggestion that applying a fur mite extract to the ears of a mouse will sensitize the mouse so that it displays at least one symptom of atopic dermatitis, as claimed. In fact, Gad is completely silent with respect to atopic dermatitis.

Hoiri et al. has been cited for its disclosure relating to testing potential therapeutics for spontaneous dermatitis in mice. That reference certainly does not remedy the deficiencies of the references discussed above.

In view of the foregoing, Claims 29, 30, and 32-37 are not *prima facie* obvious over the cited references.

In addition, the claimed mouse has an important advantage as compared to the mouse described by Morita et al. If the mouse described by Morita et al. is used for the screening of therapeutic agents, the efficacy of the agent is inevitably going to be assessed by a score as described by Hiroi et al. However, this assay is qualitative and, therefore, less accurate. On the other hand, by screening using the mouse recited in Claim 29, the efficacy of the therapeutic agent can be assessed easily and quantitatively by only measuring the thickness of the ear of the mouse.

Moreover, the mouse of the present invention has been recognized as an important contribution in the art. Applicants enclose herewith a copy of the Abstract of General Meeting of the Japanese Society of Allergology held in November 2002 and an English translation thereof. Regarding the claimed mouse, the research group of Gifu Pharmaceutical University reported the following:

> We considered this model as a severe dermatitis model with desquamation of epidermis. Repetitive stimulation by mite antigen induced Th2 elevation of Th1/Th2 balance with up-regulation of IgE production. From now on, the further analysis of pathogenesis of atopic dermatitis will be done using this model. (Presentation No. 375, Conclusion)

> The dermatitis model repeatedly applied with mite antigen on the ears of NC/Nga mice showed the Th2 dependent background. It can be considered to be useful as an animal model reflecting the one aspect of atopic dermatitis. (Presentation No. 376, Conclusion).

Thus, the research group of Gifu Pharmaceutical University reported that the claimed mouse is useful as an animal model of atopic dermatitis and that group would recognize that the claimed mouse is superior to the mouse described by Morita et al.

In view of the foregoing, Claims 29-20 and 32-37 are not obvious over the combination of the cited references. Accordingly, withdrawal of these grounds of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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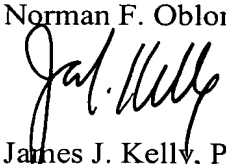
MAIER & NEUSTADT, P.C.
Norman F. Oblon



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APPENDIX

The pending claims, i.e., Claims 29, 30, and 32-37, are reproduced below.

Claims 1-28. (Canceled).

29. (Previously Amended) A mouse suitable as a model for atopic dermatitis, wherein the mouse is a NC/Nga mouse which has been sensitized with a mite extract on the ear(s) under a specific pathogen free environment, such that the animal displays at least one symptom of atopic dermatitis caused by the mite extract.

30. (Previously Amended) The mouse of Claim 29, wherein said symptom is skin lesions on the ear(s) of the mouse.

31. (Canceled).

32. (Previously Amended) The mouse of Claim 30, wherein the skin lesions are at least one member selected from the group consisting of erythema, edema, excoriation, and scaling.

33. (Previously Amended) The mouse of Claim 30, wherein the skin lesions are erythema.

34. (Previously Amended) The mouse of Claim 29, wherein the symptom is ear swelling.

35. (Previously Amended) A method of producing the mouse of Claim 29, comprising:

maintaining a NC/Nga mouse in a Specific Pathogen Free environment and sensitizing the ear(s) of the mouse with the mite extract.

36. (Previously Amended) The method of Claim 35, wherein the animal is sensitized with the mite extract for at least 5 days.

37. (Previously Amended) A method of screening for an agent for effectiveness against atopic dermatitis, comprising:

applying at least one agent to the mouse of Claim 29,

determining whether the agent reduced one or more symptoms of atopic dermatitis,

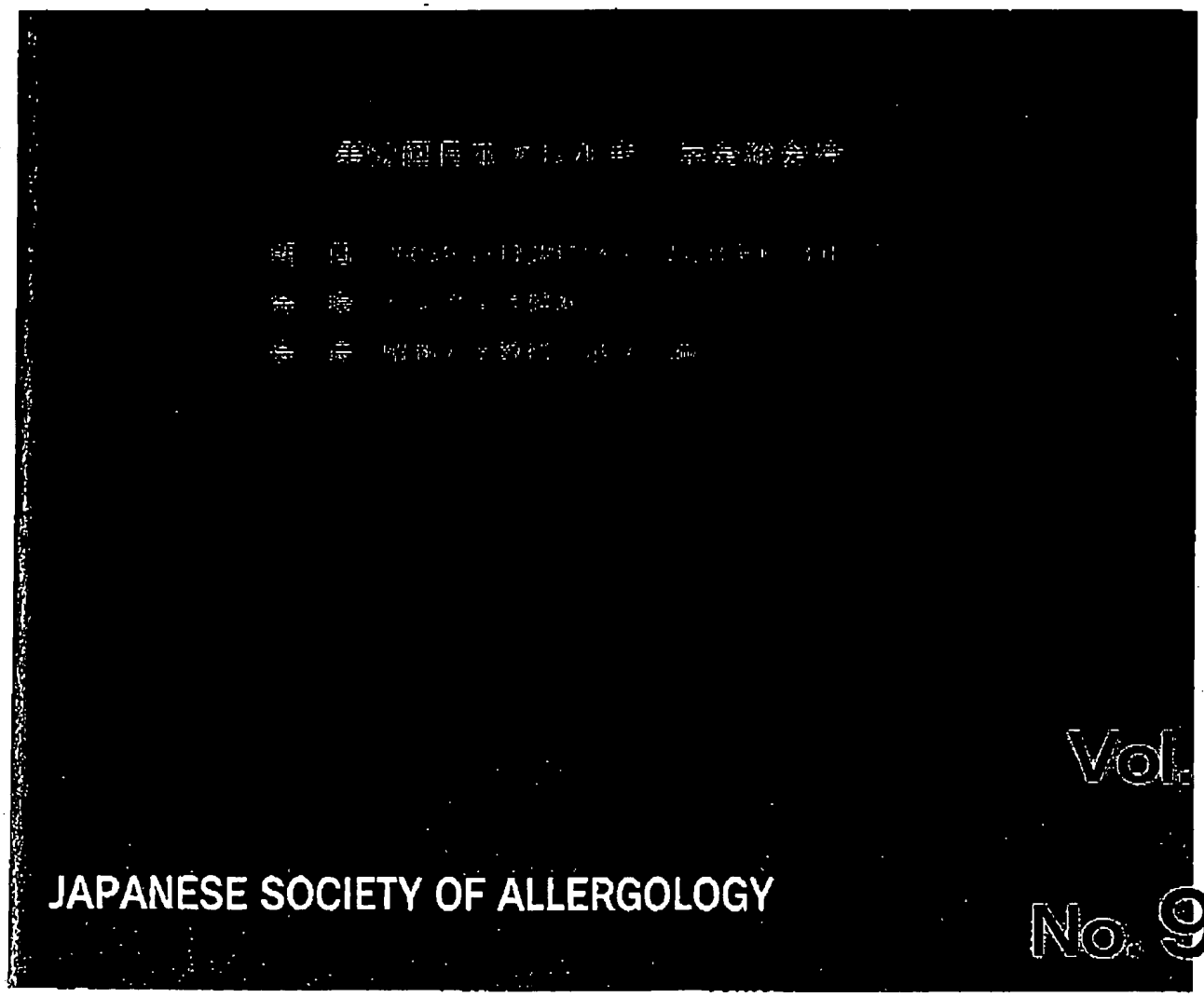
correlating a reduction in said one or more symptoms with effectiveness against atopic dermatitis, and

correlating a lack of reduction in said one or more symptoms with ineffectiveness against atopic dermatitis.

Attachment 2

アレルギー

Japanese Journal of Allergology



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373 FcεRIα 鎖特異的ヒト scFv 抗体フラグメントから Fab 抗体への抗体エンジニアリング

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昨年度のアレルギー学会でヒト FcεRIα 鎖特異的ヒト scFv 抗体フラグメントについて報告した. 今回は scFv 抗体をもとに Fab 抗体への抗体エンジニアリングを行った.
【方法と結果】pCANTAB5E ファージミドベクターに組み込まれたヒト FcεRIα 鎖特異的ヒト scFv 抗体(FcRε51)の scFv 遺伝子を鋳型として, 5'/3'末端に SfiI/SfiI サイトを設けた VH 領域増幅用のプライマー, および XhoI/NotI サイトを設けた VL 領域を増幅用のプライマーを用いて PCR を行った. 精製した VH および VL 遺伝子断片を pUC19 ベースの Fab 発現ベクターの SfiI サイトおよび XhoI/NotI サイトに順次クローニングし, Fab 発現用のベクターを作製した. 得られた発現ベクターを大腸菌 JM83 に組み込み, Fab 抗体を発現させた. この Fab 断片は, ヒト CH1 およびヒト Cκ で構成されており, H-L 間の S-S 結合部の Cys を Ser に置換してある. 単離した Fab 抗体の抗原特異性を ELISA 法で調べたところ, 可溶性 FcεRIα 鎖との結合活性が認められた. 現在この FcεRIα 特異的 Fab 抗体の性状について検討している.

374 タクロリムス軟膏はマウスの発癌を著しく促進する

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盛んに使用され始めたタクロリムス軟膏(FK506)は, 元来臓器移植の拒否反応を抑える免疫抑制剤で, 近年 FK506 を投与していた臓器移植患者千例中, CD4/CD8 の低下を伴った新しい固形癌が 7.2% の症例に発生した(Transplant, 66,1193,1998;Cancer, 80,1141,1997). 我々は 104 匹の CD-1 mice (female, 7 週齢)の背部を剃毛後, initiator, DMBA と promoter, TPA を塗布する発癌実験系(Jpn J Cancer Res, 91,579,2000)に FK506 を併用塗布して, FK506 の発癌性を検索した. 結果は, 14 w までは(1) DMBA+TPA の実験系に(2) DMBA+TPA+FK506 の系より発癌が多い傾向であったが, 14 w 以降では, (2)の系に(1)の系より著しい腫瘍の増加がみられた(0.47±0.13 vs. 0.10±0.025). また, TPA を用いない DMBA に FK506 のみを加えた系においても 26 匹中 4 匹に腫瘍が発生した(2 匹が悪性, 2 匹が良性). また, 発生した腫瘍中 8.0% が squamous cell carcinoma で, その他は papilloma であった. 以上の実験より, 14 w までは, FK506 は癌の抑制に働き, 14 w 以降は, 個体の発癌を抑制する NK cell を叩く方に作用したと推測される. 移植臓器を拒否しようとする正常な自己の NK cell, CD4 細胞を叩く薬剤は, 感染にのみならず発癌に対する自己の抵抗力も抑え, これを何年も治療を続け, 生命を脅かさぬアトピー皮膚炎に使用する事は一考を要する.

375 ダニ抗原反復塗布による皮膚炎モデルの免疫薬理学的研究—第 1 報 モデルの作製について—

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【目的】アトピー性皮膚炎(AD)は湿疹様症状と掻痒を主徴とする難治性疾患である. その病態解析にはマウスを用いた皮膚炎モデルが有用であると思われる. 本研究では BALB/c マウスと NC/Nga マウスにダニ抗原を反復塗布して生じるマウス皮膚炎モデルについて検討した. 【方法】マウスの両耳介の表裏をテープストリッピングし, ダニ抗原溶液を塗布した. ダニ抗原は週 1 回, 計 5 回塗布し, 経時的に耳介の厚さを評価した. 血清中総 IgE 値は ELISA 法を用いて, 耳介およびリンパ節におけるサイトカイン mRNA の発現は RT-PCR 法を用いて測定した. 【結果】テープストリッピングにより, 皮膚炎症状の増悪がみられた. また, ダニ抗原の 5 回目の塗布後にはいずれのマウスにも二相性の耳介腫脹が誘発された. さらに, いずれのマウスにおいても血清中総 IgE 値の上昇とともに, IL-4 mRNA 発現の増強が認められ, IFN-γ mRNA 発現は逆に低下した. 【結論】本モデルは表皮が欠損した重症の皮膚炎モデルと考えられ, 反復抗原刺激をすることにより, Th1/Th2 バランスが Th2 側へシフトして, IgE 産生の増大がみられる. 今後, 本モデルを用いて AD の病態解明, 治療薬の探索に応用したいと考えている.

376 ダニ抗原反復塗布による皮膚炎モデルの免疫薬理学的研究 —第 2 報 薬物の影響について—

布施田和紀 中村伸昭 筑本貴郎 田中宏幸
稲垣直樹 永井博弌
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【目的】アトピー性皮膚炎の病態解析および治療薬の検索にはダニ抗原誘発皮膚炎モデルが有用であると思われる. 本研究では表皮を剥離した皮膚にダニ抗原を反復塗布して作成したマウス重症皮膚炎モデルを確立し, 漢方方剤および他の薬物の影響を検討した. 【方法】NC/Nga マウスの両耳介の表裏をテープストリッピングし, ダニ抗原溶液を塗布した. ダニ抗原は週 1 回, 計 5 回塗布し, 経々の時間に耳介の厚さを測定した. 血清中総 IgE 値は ELISA 法で, 耳介およびリンパ節におけるサイトカイン mRNA の発現は RT-PCR 法で検討した. 薬物は連日経口投与した. 【結果】テープストリッピングしたマウスにダニ抗原を塗布して生じる皮膚炎は Th2 依存性が強く, 重症のアトピー性皮膚炎のモデルとなりうるものと思われた. 漢方方剤の十全大補湯は 300 mg/kg で, また, Prednisolone は 3mg/kg で耳介腫脹を有意に抑制した. 【結論】NC/Nga マウス耳介にダニ抗原を反復塗布して誘発する皮膚炎は Th2 優位な背景を持ち, アトピー性皮膚炎の一側面を反映するモデルとして有用であると思われる. また, 十全大補湯は本皮膚炎を抑制することから, アトピー性皮膚炎治療への適用の可能性が示唆される.

375 Immunopharmacological study of animal model of dermatitis with repetitive application of mite antigen – the first edition construction of animal model –

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(Department of Pharmacology, Gifu Pharmaceutical University)

[Object]
Atopic dermatitis (AD) is one of the inveterate diseases, which is mainly characterized by eczema and pruritus. Animal models of dermatitis seem to be useful for analyzing the pathogenesis of atopic dermatitis. In this study, we investigated animal models of the dermatitis caused by repetitive application with mite antigen using NC/Nga mice and BALB/c mice.
[Methods]
The ears of mice were stripped with tape, and applied with mite antigen solution five times, once a week. The ear thickeness was sequentially measured. Serum total IgE concentration was quantified with ELISA, and an expression of cytokines mRNA in the ears and lymph nodes were calculated with RT-PCR.
[Results]
The symptoms of dermatitis exacerbated by using the tape-stripping. Biphasic ear thickening was induces after the fifth application of mite antigen. In each mice, Furthermore, in each mice, as increasing of serum total IgE concentration, an expression of IL-4 mRNA was increased, whilst an expression of IFN-g mRNA were decreased.
[Conclusion]
We considerd this model as a severe dermatitis model with desquamation of epidermis. Repetitive stimulation by mite antigen induced Th2 elevation of Th1/Th2 balance with the up-regulation of IgE production. From now on, the further analysis of pathogenesis of atopic dermatitis and the screening of new drug for atopic dermatitis will be done using this model.

376 Immunopharmacological study of animal model of dermatitis with repetitive application of mite antigen – the second edition the efficacy of the drug of atopic dermatitis –

K. Fuseda, N. Nakamura, T. Chikumoto, H. Tanaka, N. Inagaki and H. Nagai
(Department of Pharmacology, Gifu Pharmaceutical University)

[Object]
Animal model of dermatitis induced by mite antigen seems to be useful for the analysis of pathogenesis of atpic dermatitis and the screening of new drug for atopic dermatitis. In this study, we established a severe dermatitis mice model by repetitive application of mite antigen on epidermis detached skin, and investigated the effect on the animal model of Chinese medicine and other drug.
[Methods]
The two sides of ears of NC/Nga mice were stripped with tape, and applied with mite antigen solution five times, once a week. The ear thickness was sequentially measured. Serum total IgE concentration was quantified with ELISA, and an expression of cytokines mRNA in the ears and lymph nodes were calculated with RT-PCR. Drugs were orally administrated every day.
[Results]
The dermatitis caused by repetitive application of mite antigen on the tape stripped mouse showed Th2 dependency, and it was thought as the model of severe atopic dermatitis. 300mg/kg of Juzen-Daiho-To, one of Chinese medicine, and 3mg/kg of prednisoline significantly suppressed the ear thickening.
[Conclusion]
The dermatitis model repeatedly applied with mite antigen on ears of NC/Nga mice showed the Th2 dependent background. It can be considered to be useful as an animal model reflecting the one aspect of atopic dermatitis. As Juzen-Daiho-To suppressed the dermatitis, it suggests that it might be effective for the human atopic dermatitis.